

CLAIMS

What is claimed is:

1. A peptide containing a specific ADMP-susceptible cleavage site.
2. A peptide of claim 1 wherein the peptide has a linking-moiety.
3. A peptide comprising a sequence of amino acids 1-40 of SEQ ID NO:1.
4. A peptide comprising a sequence of amino acids that is at least 80% identical to the sequence of amino acids 1-40 of SEQ ID NO:1.
5. A peptide of comprising a sequence of amino acids 1-40 of SEQ ID NO:2.
6. (13)A peptide comprising a sequence of amino acids 1-40 of SEQ ID NO:3.
7. (14)A peptide comprising a sequence that is at least 80% identical to the sequence of amino acids 1-40 of SEQ ID NO:3.
8. A peptide of claims 1, 2, 3, 4, 5, 6 or 7 wherein the peptide is biotinylated.
9. A peptide of claim 2 wherein the linking-moiety is a biotinylated lysine.
10. A peptide of claim 2 wherein the linking-moiety contains a chromophore.

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5 11. A peptide of claim 2 wherein the peptide
has a C-terminal linking-moiety.

12. A peptide of claim 2 wherein the peptide has a C-
terminal linking-moiety that is a biotinylated
10 lysine.

13. A peptide of claim 2 wherein the peptide
has an N-terminal linking-moiety.

15 14. A peptide of claim 2 wherein the peptide has an N-
terminal linking-moiety that is a biotinylated
lysine.

15. A product peptide of claim 1 comprising the amino
20 acids from the N-terminus through P1 of the ADMP-
susceptible cleavage bond.

16. A product peptide of claim 1 comprising the amino
acids from P1' of the ADMP-susceptible cleavage bond
25 through C-terminus.

17. A peptide of claims 15 or 16 wherein the peptide
is biotinylated.

30 18. A peptide of claim 15 wherein the peptide has an N-
terminal linking-moiety.

19. A peptide of claim 16 wherein the peptide has a C-
terminal linking-moiety.

35 20. A peptide of claim 18 wherein the linking-
moiety is a biotinylated lysine.

5 21. A peptide of claim 19 wherein the linking-
moiety is a biotinylated lysine.

22. A peptide of claim 18 wherein the linking-
moiety contains a chromophore.

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23. A peptide of claim 19 wherein the linking-
moiety contains a chromophore.

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24. A peptide comprising a sequence of amino acids 20-40
of claim 3, wherein an additional biotinylated
lysine is attached to the C-terminus via a peptide
bond, comprising a sequence of amino acids of SEQ ID
NO:5.

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25. A peptide comprising a sequence of amino acids 1-20
of claim 3, wherein an additional biotinylated
lysine is attached to the N-terminus via a peptide
bond, comprising a sequence of amino acids of SEQ ID
NO:6.

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26. A method for the determination of the presence of
aggrecan-degrading metalloprotease activity
comprising:

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(a) binding an ADMP substrate peptide of claim
1 to a streptavidin-coated microtiter
plate;

(b) rinsing the microtiter plate with assay
buffer;

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(c) incubating the microtiter plate with an
ADMP-containing sample;

(d) rinsing the microtiter plate;

(e) incubating the microtiter plate with a
neoepitope antibody solution;

(f) rinsing the microtiter plate;

- 5 (g) incubating microtiter plates with
secondary-detection antibody solution;
(h) incubating the microtiter plate with an
appropriate substrate solution;
(i) quenching the reaction;
10 (j) reading the optical density;
27. The method of claim 26, wherein said ADMP peptide
substrate comprises a covalently-linked linking-
moiety.
- 15 28. A method for the determination of ADMP activity by
quantifying the appearance of a product peptide
comprising:
- 20 (a) incubating an ADMP substrate peptide of
claim 1 with assay buffer and ADMP-
containing sample;
(b) quenching the reaction;
(c) injecting a portion of the reaction mixture
onto a reverse-phase HPLC column;
25 (d) eluting the peptide with an organic
solvent;
(e) reading the absorbance;
(f) determining the quantity based on a
standard curve.
- 30 29. A method for assaying compounds for activity against
an ADMP comprising:
- 35 (a) providing an ADMP and an ADMP substrate;
(b) contacting said ADMP with a candidate
inhibitory compound in the presence of
said ADMP; and
(c) measuring the inhibition of the ADMP
activity.

- 5 30. A method for assaying compounds according
to claim 29 wherein the ADMP activity is monitored
according to claim 26 or 28.
- 10 31. A peptide of claim 3, 4, or 5 wherein the P1 amino
acid residue, Glu, the ADMP-sensitive Glu373-Ala374
bond, is esterified.
- 15 32. A peptide of claim 3, 4, or 5 wherein the P1 amino
acid residue, Glu, of the ADMP-sensitive Glu373-
Ala374 bond, is replaced with a Gln amino acid
residue.
- 20 33. An assay for detecting ADMP activity which comprises:
(a) incubating a sample containing
soluble ADMPs or aggrecanase activity with
an aggrecan substrate; and
(b) monitoring production of
aggrecan fragments produced by specific
cleavage at an ADMP-susceptible site using
a neoepitope antibody to the new N-
terminus or the new C-terminus generated
by specific ADMP-mediated cleavage by the
Problot assay comprising:
(1) incubate a
30 polyvinyl-
denedifluoride (PVDF) cationically
charged membrane, secured in a well
filtration plate, with a sample
containing ADMP-degraded aggrecan;
35 (2) wash any unbound aggrecan from
the filtration plate;
(3) couple any unreacted cationic
sites on the PVDF membrane with a

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5 solution of bovine serum albumin
(BSA);
(4) wash any unbound BSA from the
filtration plate;
(5) remove glycosaminoglycan side
10 chains from the bound aggrecan with
deglycosylation enzymes, wash
membrane;
(6) incubate PVDF membrane with a
neoepitope antibody to fragments
generated by cleavage at an ADMP-
15 sensitive site, wash membrane;
(7) incubate PVDF membrane with
secondary detection antibody, wash
membrane;
(8) incubate PVDF membrane with
20 detection substrate;
(9) drain solution into wellled plate,
obtain absorbance readings on
individual samples; compare values to
those obtained for standard curve.
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34. A method for assaying compounds according to claim 29
wherein ADMP activity is monitored according to
claim 33.

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35. An assay according to claim 33 wherein the tissue
or cell source of ADMPs is cartilage or
chondrocytes.

35 36. An assay according to claim 33 or 34 wherein the
aggrecan substrate is native aggrecan isolated from
human or animal tissue.

- 5 37. An assay according to claim 33 or 34 wherein the
aggrecan substrate is a recombinant aggrecan
molecule or recombinant portion of the aggrecan
molecule containing an aggrecanase-sensitive
cleavage site.
- 10 38. An assay according to claim 33 or 34 wherein the
recombinant portion of the aggrecan molecule
contains the E³⁷³⁻³⁷⁴A bond.
- 15 39. An assay according to claim 33 or 34 wherein the
recombinant aggrecan fragment contains the E¹⁵⁴⁵⁻¹⁵⁴⁶G
bond.
- 20 40. An assay according to claim 33 or 34 wherein the
portion of the aggrecan molecule contains the E¹⁷¹⁴⁻¹⁷¹⁵G bond.
- 25 41. An assay according to claim 33 or 34 wherein the
recombinant portion of the aggrecan molecule
contains the E¹⁸¹⁹⁻¹⁸²⁰A bond.
- 30 42. An assay according to claim 33 or 34 wherein the
recombinant portion of the aggrecan molecule
contains the E¹⁹¹⁹⁻¹⁹²⁰L bond.
- 35 43. A method according to claims 26, 30, 33, or 34
wherein the neoepitope antibody recognizes the new
N-terminus or new C-terminus generated by cleavage
at the E³⁷³⁻³⁷⁴A bond.
44. A method of any of claims 26, 30, 33, or 34 wherein
the neoepitope antibody is the BC-3 monoclonal
antibody.

- 5 45. A method of any of claims 26, 30, 33, or 34
wherein the neoepitope antibody recognizes the new
N-terminus or new C-terminus generated by cleavage
at the E1545-G1546 bond.
- 10 46. A method of any of claims 26, 30, 33, or 34
wherein the neoepitope antibody recognizes the new
N-terminus or new C-terminus generated by cleavage
at the E1714-G1715 bond.
- 15 47. A method of any of claims 26, 30, 33, or 34 wherein
the neoepitope antibody recognizes the new N-
terminus or new C-terminus generated by cleavage at
the E1819-A1820 bond.
- 20 48. A method of any of claims 26, 30, 33, or 34 wherein
the neoepitope antibody recognizes the new N-
terminus or new C-terminus generated by cleavage at
the E1919-L1920 bond.
- 25 49. A method of use of the assay in claim 33 for
detecting ADMP-generated aggrecan fragments in
culture media from tissue or cell cultures
stimulated to induce aggrecanase-mediated
degradation.
- 30 50. A method of use of the assay in claim 33 for
detecting aggrecanase-generated aggrecan fragments
in biological fluids, tissue extracts or
homogenates, serum or urine from patients with
35 aggrecanase-associated diseases.
51. A method for diagnosing arthritic diseases in a
mammal by monitoring ADMP-generated aggrecan
fragments according to claims 33.

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52. A method for diagnosing a disease in a mammal
characterized by overproduction or up-regulated
production of an ADMP by monitoring fragments
generated at an ADMP-sensitive site according to
claims 33.

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